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FURTHER OBSERVATIONS ON THE USE OF PNEUMOCOCCUS EXTRACTS IN EFFECTING TRANSFORMATION OF TYPE IN VITRO

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The fact has been conclusively proved by Griffith (1), and subsequently confirmed by Neufeld and Levinthal (2) and Dawson (3) that R pneumococci derived from S organisms of one specific type may be transformed into S forms of a different specific type. The change was first accomplished *in vivo* by Griffith, who injected into mice living R organisms derived from one type of Pneumococcus together with large quantities of heat-killed S pneumococci of a different type. Living S forms of the same type as that of the killed S organisms injected, were recovered from the animals.

Dawson and Sia (4, 5) succeeded later in bringing about transformation of type *in vitro*. They inoculated with minute quantities of living R organisms derived from one type of Pneumococcus a medium of broth containing anti-R serum and the whole heat-killed S organisms of a different type. From the cultures originally inoculated with R pneumococci, S organisms were recovered which were identical in type with that of the killed S pneumococci present in the medium. In a previous paper from this laboratory (7), experiments were described in which transformation of type had been effected *in vitro* through the use of cell-free extracts of S pneumococci. These extracts, although filtrable, were crude and contained large amounts of cellular debris now known to be unessential for the transformation. Moreover, the earlier method of preparation resulted in the loss of a considerable amount of the active substance. The present paper describes a more efficient method of extracting pneumococci, and records the results of attempts to purify further the active substance.

Methods

Cultures.—The cultures of R pneumococci used in the experiments were stock strains originally derived from type-specific S pneumococci by growth in broth containing 10 per cent antipneumococcus serum of the homologous type. The pneumococci from which the extracts were prepared were type-specific S strains, the virulence of which had been maintained by frequent animal passages.

Preparation of Pneumococcus Extract.—The S pneumococci used in preparing extracts were grown in meat infusion broth, pH 7.8, containing dextrose 0.01 per cent. Large inocula were used to bring about rapid and dense growth. At least 100 cc. of a moderately heavy culture was used to inoculate 5 liters of media. The cultures were grown at 37°C. for 8 to 10 hours only. The organisms from 5 liters of culture were thrown down and then taken up in 50 cc. of sterile distilled water. To this suspension was added 3.5 cc. of a sterile 10 per cent solution of sodium desoxycholate. The mixture was kept in an ice bath for 10 minutes, then brought slowly to 60°C. in a water bath. The organisms treated in this manner are quickly dissolved, forming a thick, extremely viscous gel. The preparation was kept in the water bath at 60°C. for 15 minutes to kill any surviving organisms, and to inhibit or destroy the autolytic enzymes released by dissolution of the cells. The bacterial solution was added slowly to 500 cc. of absolute alcohol previously chilled in an ice bath. A thick, stringy precipitate formed which slowly settled out on standing. The sodium desoxycholate, being soluble in alcohol, remained in the supernatant fluid. The precipitate was thrown down after 30 minutes, by centrifugation, the supernatant fluid being discarded. The sodium desoxycholate solution was thus eliminated. The precipitate was washed with alcohol to remove the last traces of the bile salt and was dried *in vacuo*, or by exposure for 12 to 14 hours to cold dry air, The dried material was then extracted in 100 cc. of sterile 0.85 per cent sodium chloride solution, made slightly alkaline by the addition of 0.5 cc. of N/10 sodium hydroxide, sealed in glass ampoules, and immersed for 15 minutes in a water bath at 60°C. The preparation was then centrifuged for 30 minutes and the insoluble residue, which contained only a small amount of the active material, was discarded. The supernatant extract was a slightly turbid, thin, opalescent fluid. The extracts were subjected to rigid tests for sterility. They were cultured in plain broth, in blood broth, on blood agar, and in media enriched by the addition of ascitic or chest fluid. In no instance were pneumococci grown from the extracts. Injected in amounts as large as 1 cc. into mice, they caused no untoward effects other than slight lethargy for a few hours. All the test animals survived.

Culture Medium.—Dawson and Sia found in their *in vitro* experiments (4, 5), that the transformation of pneumococci from one specific type to another was facilitated by the addition to the culture medium of anti-R serum. Their observations have been repeatedly confirmed

in this laboratory. In fact, no transformation has been possible in the author's experience without the use of serum or a serous fluid in the medium.

Hog serum and anti-R rabbit serum were used in earlier experiments. It has been found recently, however, that sterile ascitic or chest fluid is more effective than blood serum, when added to the nutrient broth in proportion of 1 to 3. The chest fluid used in the present experiments was obtained from a patient with cardiac failure and general anasarca. It was a straw-colored, clear transudate, and proved sterile on culture. Prior to use it was filtered through a Berkefeld V candle. Titrations of its anti-R properties revealed that it would agglutinate R pneumococci only in dilutions of 1–20, or, rarely, 1–40.

Cultural Technic.—The cultural technic employed in effecting the transformations was similar, save for minor changes, to that described in an earlier paper (7). Inocula, consisting of 1 drop of a 10^{-4} dilution of an 8 hour culture of R pneumococci, were added to a series of tubes containing 1.5 cc. of broth, 0.5 cc. of chest fluid, and varying quantities, 0.05 to 1.0 cc., of the specific pneumococcus extract. The cultures were grown aerobically at 37°C. for 24 hours. Transfers to fresh medium were made serially every 24 hours, 1 drop of culture being carried forward to a corresponding tube in the second series. At the time of each transfer, subcultures were made on blood agar plates for the subsequent study of the colony characteristics of the organisms. No experiment was considered negative until five serial transfers were made. Whenever smooth colonies were noted on subculture, one typical colony was transferred to blood broth and the specific type of the organisms was subsequently identified serologically.

Activity of Extracts.—Extracts made from S pneumococci by the action of sodium desoxycholate proved much more effective in inducing transformations in type than did the extracts which were formerly prepared from frozen and thawed organisms. They were effective in high dilution, and even failed to induce a change if present in too great concentration. Conversion of R pneumococci derived from Type II S organisms into Type III pneumococci could be accomplished in almost all instances by means of an extract of Type III organisms. Table I shows the results of a typical experiment.

Further Purification of Extracts

Adsorption on Charcoal.—Attempts were made to separate the active transforming material from the inert cellular debris by the use of various adsorbents. Powdered wood charcoal was found to give

satisfactory and consistent results. Purification with charcoal was carried out as follows:

100 cc. of the extract prepared in the manner described was diluted with an equal volume of sterile 0.85 per cent solution of sodium chloride. 8 gm. of sterile powdered wood charcoal was added to the diluted extract and the mixture was shaken for 5 minutes. The preparation was then centrifuged at high speed for 30 minutes and the supernatant fluid, still containing considerable amounts of finely

TABLE I

Activity of Extract of S Pneumococci before Adsorption and Purification

Tube	Broth	Serum factor (chest fluid)	R organism	Specific extract Type III pneumococci*	Colonies	Specific agglutination of S colonies
			First culture			
	cc.	*cc.*		*cc.*		
1a	1.5	0.5	D-39-R†	0.1	R and S	Type III
2a	1.5	0.5	D-39-R	0.1	R only	—
3a	1.5	0.5	D-39-R	0.05	R only	—
4a	1.5	0.5	D-39-R	0.05	R only	—
5a	1.5	0.5	—	0.1	Sterile	—
			First subculture			
1b	1.5	0.5	From Tube 1a	0.1	R and S	Type III
2b	1.5	0.5	From Tube 2a	0.1	R and S	Type III
3b	1.5	0.5	From Tube 3a	0.05	R and S	Type III
4b	1.5	0.5	From Tube 4a	0.05	R only	—

* Pneumococcus extract prepared by dissolving Type III S organisms in a solution of sodium desoxycholate with removal of bile salt by precipitating the extract in alcohol.

† Strain of R Pneumococcus derived from Type II S organisms.

suspended charcoal, was removed. This solution was filtered through sterile filter paper and finally through a Berkefeld V candle. The filtered extract was generally water-clear, colorless, and quite limpid.

Extracts purified by the use of charcoal were more active in effecting transformation than were the crude extracts. Moreover, it was much easier to determine when transformation had occurred in cultures containing these clear extracts. The medium was entirely clear and transparent on inoculation. Growth was granular and settled

out at the bottom of the tube as long as the organisms retained their R characteristics. When S cells developed, however, growth became diffuse throughout the medium. The presence of diffuse growth in the culture medium was presumptive evidence that S colonies would be found later on plating.

TABLE II

Activity of Pneumococcus Extract after Charcoal Adsorption

Tube	Broth	Serum factor (chest fluid)	R strain of Pneumococcus derived from Type:	Specific extract prepared from S pneumococci*		Colonies	Specific agglutination of S colonies
				Type	Amount		
First culture							
	cc.	*cc.*			*cc.*		
1a	1.5	0.5	I	I	0.75	R and S	Type I
2a	1.5	0.5	I	II	0.75	R only	—
3a	1.5	0.5	I	III	0.75	R only	—
4a	1.5	0.5	II	I	0.75	R only	—
5a	1.5	0.5	II	II	0.75	R and S	Type II
6a	1.5	0.5	II	III	0.75	R and S	Type III
7a	1.5	0.5	III	I	0.75	R and S	Type III
8a	1.5	0.5	III	II	0.75	R only	—
9a	1.5	0.5	III	III	0.75	R and S	Type III
10a	1.5	0.5	—	I	1.0†	Sterile	—
11a	1.5	0.5	—	II	1.0	Sterile	—
12a	1.5	0.5	—	III	1.0	Sterile	—
First subculture							
2b	1.5	0.5	From Tube 2a	II	0.75	R only	—
3b	1.5	0.5	From Tube 3a	III	0.75	R and S	Type III
4b	1.5	0.5	From Tube 4a	I	0.75	R and S	Type I
8b	1.5	0.5	From Tube 8a	II	0.75	R and S	Type II
Second subculture							
2c	1.5	0.5	From Tube 2b	II	0.75	R and S	Type II

* Extracts prepared by dissolving S pneumococci in a solution of sodium desoxycholate, precipitating in alcohol, redissolving in salt solution, adsorbing on charcoal, and filtering through Berkefeld V candle; Type I extract prepared from Type I pneumococci, Type II from Type II pneumococci, and Type III from Type III pneumococci.

† Controls were prepared using 1.0 and 0.5 cc. quantities of the extract in the original experiment which is shown here much abridged. All controls were sterile.

Charcoal-adsorbed extracts of Types I, II, and III S pneumococci were prepared and found to be highly active in all instances. In Table II are recorded the transformations brought about through the use of extracts after removal of considerable inactive material by charcoal adsorption.

As the results presented in Table II show, it was possible to cause R pneumococci derived from each of the three specific types to revert to their original S forms through the use of specific extracts of the homologous type. Likewise, it was possible, with one exception, to effect a selective transformation in type, whereby the R cells derived from each type of Pneumococcus were changed into the S forms of each of the other two specific types in the presence of the appropriate extract. It was impossible, in two attempts made, to effect transformation of R pneumococci derived from Type III S organisms into Type I pneumococci. Instead, these particular R pneumococci reverted to S forms of the original specific type from which they were derived; namely, Type III. The tendency of R pneumococci to revert to S organisms of the original type, even in the presence of a suspension of heat-killed S organisms of a heterologous type, was encountered and commented upon both by Griffith and by Dawson.

As stated in the previous paper (7), it was quite easy to convert R pneumococci derived from Type II S organisms into Type III S forms. In most instances the change occurred in the first culture within 15 to 20 hours. Other R strains changed less readily and often required two, three, and even four transfers. Thus, in the experiment shown in Table II, the R organisms derived from Type I S pneumococci were changed into Type II forms only on the third successive cultivation in the extract-containing medium.

Reprecipitation in Acetone or Alcohol.—Still further purification of the charcoal-adsorbed extracts was accomplished by precipitation in acetone.

The filtered extract, after charcoal adsorption, was precipitated by 10 volumes of acetone in the cold. The preparation, after standing 1 hour, was centrifuged at high speed for 30 minutes and the supernatant acetone discarded. The sediment was dried *in vacuo* and taken up in the original volume of sterile distilled water. Much of the precipitate remained insoluble. The insoluble residue was thrown down by centrifugation and discarded. The supernatant contained the active material without appreciable loss.

Alcohol was substituted for acetone with equal success. Most of the active material was precipitated by 70 per cent alcohol: all by 100 per cent. The active substance after precipitation in acetone or alcohol was soluble in water. No demonstrable loss in potency resulted from treatment with these reagents as is evidenced by the data presented in Table III.

Properties of the Extract

Resistance to Heat.—The transforming substance was more resistant to heat in extracts freed from much of the extraneous material present in the whole pneumococcus cell and in the crude preparations. Griffith and Dawson both found (1, 3, 6) that heating the intact cells

TABLE III

Activity of Pneumococcus Extract after Precipitation by Acetone

Tube	Broth	Serum factor (chest fluid)	R Pneumococcus derived from Type II organisms	Specific extract of Type III pneumococci*	Colonies	Specific agglutination of S colonies
	cc.	cc.		cc.		
1	1.5	0.5	D-39-R	1.0	R and S	Type III
2	1.5	0.5	D-39-R	1.0	R and S	Type III
3	1.5	0.5	D-39-R	0.5	R and S	Type III
4	1.5	0.5	—	1.0	Sterile	—

* Pneumococcus extract prepared by precipitating the charcoal-adsorbed purified extract of S organisms in acetone and extracting the precipitate in distilled water.

to temperatures above 80°C. rendered the bacterial suspensions used in effecting transformations *in vivo*, incapable of inducing changes in type, whereas heating them at lower temperatures, if the exposure was not too long, caused very little decrease in potency. Experiments with purified extracts demonstrated that although these preparations showed a progressive drop in potency on heating above 80°C., nevertheless they were occasionally active after 10 minutes' exposure in the water bath to a temperature as high as 90°C. The effect on the activity of an extract heated for 10 minutes at 70°, 80°, and 90°C., respectively, is evident from the experimental results given in Table IV.

TABLE IV

Effect of Heat on the Activity of Purified Pneumococcus Extract

Tube	Broth	Serum factor (chest fluid)	R organism	Type III specific extract*		Colonies	Specific agglutination of S colonies
				Heated for 10 min. at	Amount		
First culture							
	cc.	*cc.*		°C.	*cc.*		
1a	1.5	0.5	D-39-R†	70	1.0	R and S	Type III
2a	1.5	0.5	D-39-R	70	1.0	R only	—
3a	1.5	0.5	D-39-R	70	0.5	R only	—
4a	1.5	0.5	D-39-R	80	1.0	R only	—
5a	1.5	0.5	D-39-R	80	1.0	R only	—
6a	1.5	0.5	D-39-R	80	0.5	R and S	Type III
7a	1.5	0.5	D-39-R	90	1.0	R only	—
8a	1.5	0.5	D-39-R	90	1.0	R only	—
9a	1.5	0.5	D-39-R	90	0.5	R only	—
First subculture							
1b	1.5	0.5	From Tube 1a	70	1.0	R and S	Type III
2b	1.5	0.5	From Tube 2a	70	1.0	R only	—
3b	1.5	0.5	From Tube 3a	70	0.5	R and S	Type III
4b	1.5	0.5	From Tube 4a	80	1.0	R and S	Type III
5b	1.5	0.5	From Tube 5a	80	1.0	R and S	Type III
6b	1.5	0.5	From Tube 6a	80	0.5	R and S	Type III
7b	1.5	0.5	From Tube 7a	90	1.0	R only	—
8b	1.5	0.5	From Tube 8a	90	1.0	R and S	Type III
9b	1.5	0.5	From Tube 9a	90	0.5	R only	—
Second subculture							
2c	1.5	0.5	From Tube 2b	70	1.0	R and S	Type III
7c	1.5	0.5	From Tube 7b	90	1.0	R only	—
8c	1.5	0.5	From Tube 8b	90	1.0	R and S	Type III
9c	1.5	0.5	From Tube 9b	90	0.5	R only	—
Third subculture							
7d	1.5	0.5	From Tube 7c	90	1.0	R only	—
9d	1.5	0.5	From Tube 9c	90	0.5	R only	—

* Extract of Type III S pneumococci prepared by dissolving the culture in a solution of sodium desoxycholate, precipitating in alcohol, and extracting the precipitate in saline solution.

† Strain of R Pneumococcus derived originally from Type II S organisms.

From the data presented in Table IV, it is apparent that pneumococcus extracts became progressively less active after exposure to temperatures above 80°C., and that the loss incurred was approximately in direct proportion to the degree of heating. However, it is also evident that in the present state of purity, an extract occasionally retained sufficient activity to bring about changes even after heating to 90°C. for 10 minutes. Boiling invariably destroyed the activity of the purified extracts.

TABLE V

Activity of Purified Pneumococcus Extract after Filtration through Berkefeld W Candle

Tube	Broth	Serum factor (chest fluid)	R organism	Specific extract prepared from Type III S*	Colonies	Specific agglutination of S colonies
			First culture			
	cc.	*cc.*		*cc.*		
1a	1.5	0.5	D-39-R†	1.0	R only	—
2a	1.5	0.5	D-39-R	1.0	R only	—
3a	1.5	0.5	D-39-R	0.5	R only	—
4a	1.5	0.5	D-39-R	0.5	R only	—
5a	1.5	0.5	D-39-R	0.25	R only	—
6a	1.5	0.5	—	0.5	Sterile	—
			First subculture			
1b	1.5	0.5	From Tube 1a	1.0	R and S	Type III
2b	1.5	0.5	From Tube 2a	1.0	R and S	Type III
3b	1.5	0.5	From Tube 3a	0.5	R and S	Type III
4b	1.5	0.5	From Tube 4a	0.5	R and S	Type III
5b	1.5	0.5	From Tube 5a	0.25	R only	—

* Extract of Type III pneumococci prepared by filtration of purified, charcoal-adsorbed extract through Berkefeld W candle.

† Strain of R Pneumococcus derived from Type II S.

Filtrability.—It was stated in a previous paper (7) that pneumococcus extracts prepared by freezing and thawing the bacterial cells, could be filtered through Berkefeld N candles and still retain their capacity for effecting changes in type. Recent investigations showed that when the purified extract was alkaline in reaction, filtration could be carried out without demonstrable loss of potency, not only

through the N but also through the W type of Berkefeld filter. When the extract was acid, however, filtration resulted in complete loss of activity. The purified extracts passed through W filters were crystal-clear and colorless. They still exhibited full activity as demonstrated by the results of the filtration experiment recorded in Table V.

TABLE VI

Active Immunity Induced in Mice by Injection of Purified Pneumococcus Extract (Type I)

Mouse	Type I extract* injected†			Virulent culture of Type I pneumococci injected† May 11	Result
	Apr. 27	Apr. 30	May 4		
	cc.	*cc.*	*cc.*	*cc.*	
1	0.2	0.2	0.2	0.001	S
2	0.2	0.2	0.2	0.001	D 18
3	0.2	0.2	0.2	0.0001	D 18
4	0.2	0.2	0.2	0.0001	S
5	0.2	0.2	0.2	0.00001	S
6	0.2	0.2	0.2	0.00001	S
7	0.2	0.2	0.2	0.000001	S
8	0.2	0.2	0.2	0.000001	D 60

Virulence Control

Mouse	Virulent Type I culture†	Result
	cc.	
1	0.001	D 18
2	0.0001	D 18
3	0.00001	D 24
4	0.000001	D 40
5	0.0000001	D 40
6	0.00000001	D 40

* Extract prepared by dissolving Type I pneumococci in a solution of sodium desoxycholate, precipitating in alcohol, extracting the precipitate in saline solution, adsorbing with charcoal, and filtering through Berkefeld V candle.

† All injections made intraperitoneally.

S = survived; period of observation 21 days.

D = died; the numeral indicates the number of hours before death of the animal.

Soluble Specific Substance.—The purified extracts contained varying amounts of the soluble specific substance of the pneumococci from which they were prepared. However, the concentration of the type-

specific substance was relatively low, since in most instances the purified extracts reacted specifically in antipneumococcus serum of the homologous type only in dilutions of 1–40, or occasionally 1–80. This indicates a probable concentration of the specific capsular polysaccharide of approximately 0.01 mg. per cc. of extract.

Antigenicity.—It was of interest to learn whether these extracts possessed antigenic as well as transforming properties. It was found that the extracts as prepared were not only active in effecting transformation in type, but also were capable of inducing active immunity in mice treated with the purified preparations. The protocol shown in Table VI illustrates the degree of active immunity induced in mice by repeated intraperitoneal injections of an active extract.

From the results recorded in Table VI, it can be seen that considerable active immunity was induced in mice by three injections, at 3 day intervals, of 0.2 cc. of purified extract of Type I pneumococci. Of 8 mice so treated and inoculated 7 days later with a strain of virulent Type I Pneumococcus, 5 survived an infecting inoculum 100 to 100,000 times greater than that which invariably proved fatal to the normal control animals.

DISCUSSION

Extracts of S pneumococci prepared by the dissolving action of sodium desoxycholate were as active as were the intact cells themselves in causing R forms to assume type-specific characters. Their action was specific, the change in type being selectively determined by the specificity of the extract employed. The active substance or substances in the crude extract inducing the changes could be considerably freed from accompanying impurities by precipitation in alcohol, by charcoal adsorption, and reprecipitation in alcohol or acetone. These procedures seemed not to decrease the potency of the active preparations. In fact, on further purification, the extracts exhibited in most instances increased activity, inducing more prompt transformation.

Despite the fact that the capsular polysaccharide of the Pneumococcus determines its type specificity, it was not possible to correlate the activity of the extracts which stimulate the development of type-specific characteristics in R pneumococci, with the presence of

the soluble specific substance. Extracts which were apparently equally potent in causing transformation varied considerably in their content of soluble specific substance. It has been proved (6) that the specific capsular polysaccharide in chemically purified form, as such, is ineffective in inducing transformation in type. It seems probable therefore, that if the soluble specific substance in these extracts is concerned at all in the reaction, it is present there in a different physical state, or in combination with some other substance which confers upon it properties not found in the chemically isolated and highly purified substance.

Certain strains of R pneumococci were found to be more resistant to transformation than were others, but none were encountered which were completely refractory. Transformation of R pneumococci derived from Type II strains to the Type III S forms in the presence of Type III extract seemed to take place almost abruptly. Very rarely were transitional colonies noted. Likewise, under the influence of Type II extract, R cells derived from Type III organisms changed abruptly to Type II pneumococci. However, the change of R forms derived from Type II pneumococci to Type I S organisms was a more gradual one, and required, at times, a series of transfers in the specific extract medium. In this instance, all stages in transformation were noted in colonies plated from an individual culture. Once the change was complete, however, the newly acquired type-specific characters persisted.

The factor which is presumably common to blood serum, ascitic and chest fluid, and which is essential in the reaction, remains unknown. No experiments were successfully completed without the addition to the medium of one or another of these three related substances.

The present experiments afford additional evidence that the transformation in type is not apparent, but real, and that the changes are brought about in the presence of the extract through the specific action of a soluble constituent present in S forms of pneumococci. It is almost inconceivable that any living element in the pneumococcus cell could survive the drastic procedures employed in the preparation of the extracts. Through the action of sodium desoxycholate, pneumococci were completely dissolved so that no recognizable cellular forms remained. The extracts were heated to 60°C. for a total of 30

minutes during the course of preparation, and in sealed glass tubes were completely immersed in the water bath during part of the heating. They were exposed to the action of absolute alcohol for 30 minutes, and were saturated with alcohol of varying strength for several hours. They were treated with charcoal which removed much of the particulate matter, and were finally filtered through Berkefeld V filters. They could even be heated to 90°C. for short periods, or passed through Berkefeld W filters and still remain active. Controls of sterility were exceedingly rigid. The extracts were injected in large amounts into mice without any untoward effects. All cultures of the extracts and of animals sacrificed at various intervals after the injection of active material, were sterile.

The exact nature of the active material in these extracts still remains to be determined. That it acts as a specific stimulus to the R cells which have potentially the capacity of elaborating the capsular polysaccharides of any one of the several types of pneumococci seems clear.

SUMMARY

Pneumococcus extracts highly active in inducing the *in vitro* transformation of the specific types of Pneumococcus have been prepared by dissolving S cells with sodium desoxycholate, precipitating the dissolved material in alcohol in which the bile salt remains soluble, and extracting the precipitate in salt solution. Further purification of these active extracts has been attained by the removal of considerable inactive material by charcoal adsorption and by reprecipitation of the adsorbed extract in alcohol or acetone. The importance of using young cultures for extraction, and of preventing autolysis during the preparation of the extracts, is emphasized.

Extracts prepared by the method described have been filtered through Berkefeld Candles (V, N, and W) without appreciable loss in activity, provided the reaction of the extract was slightly alkaline at the time of filtration. The purified and filtered extracts are water-clear, and sterile by rigid cultural and animal tests. They have been heated to temperatures of 60°C. for 30 minutes without appreciable loss in their capacity to induce specific changes in type. And although they have generally shown definite decrease in potency after

heating to temperatures above 80°C., some extracts have been found active even after an exposure of 10 minutes to a temperature of 90°C. They have been completely inactivated by boiling.

Relatively small amounts of extract have been effective when added to a broth medium containing normal serum or serous fluid. In this medium, R pneumococci, irrespective of their type derivation, have developed and thereafter retained all the type-specific characteristics of the encapsulated S cells from which the extract was prepared.

The specific action of the extracts is discussed with reference to their transforming and antigenic properties.

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